Biocriteria for Puget Sound: A Summary of Phases I Through III

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Introduction

Development of biocriteria depends on the premise that population and condition parameters of marine biota provide a sensitive screening tool for assessing the condition of a water resource (M.L. Bowman et. al., 1997). The purpose of the biocriteria effort is to incorporate biotic health and ecological metrics derived from the sampling of multiple biological assemblages into a broad-based index using natural reference conditions as benchmarks—the biocriteria. Once biocriteria are developed, based on minimally impaired reference conditions, sites are evaluated to determine how well they measure up against the criteria. The greater the discrepancy, the greater the potential impairment of the water resource. The biocriteria should be carefully developed so as to closely represent the natural biota, provide the sensitivity to identify marginally disturbed sites, protect areas against further degradation, and stimulate restoration of degraded sites. These biological measures should be based on sound scientific principles that are quantifiable and written to protect or enhance the designated use. To account for a measure's natural variability in a healthy environment, the criterion should be designed to accommodate seasonality and should be defined as a range rather than as a discrete value, often represented graphically as box plots. By linking the assessment and cleanup of environmental impairment to a biological index, the goal is to make the evaluation process not only much more meaningful—both socially and ecologically—but also much more economical.

The three phases of the Puget Sound biocriteria pilot study (Eaton and Dinnel 1993; Eaton 1994, 1995) is a part of the national developmental research effort funded by EPA headquarters designed to construct meaningful biocriteria/bioassessment metrics for measuring environmental impairment and cleanup goals. Two years of research were conducted in the Tacoma area in 1993 and 1994 (Phases I and II) and in Sinclair Inlet, Port Orchard and Quartermaster Harbor in 1997 (Phase III) to develop and assess the sampling methods for three biological assemblages: demersal fish, epibenthic macroinvertebrates, and macrobenthos. The pilot studies assessed the utility of using two different trawls (the 7.6-m otter trawl and the 3-m beam trawl) and a 0.2-m^2 van Veen sediment sampler with multiple sample replications to define demersal populations of marine fishes and epibenthic and infaunal invertebrates. Using the documented population patterns and comparisons between reference and contaminated stations, the study objectives were ultimately to:

- gain a greater understanding of how demersal populations are being affected by pollution and habitat degradation;
- determine which biological patterns reflect environmental stress; and
- develop prototype biological metrics from the data that would help to build a biological index for the rapid and economical assessment of stress in subtidal biotic marine communities.

Quantitative methods of economically sampling three different biological assemblages representing three different scales of analysis have now been developed:

1. Demersal fishes using the 7.6-m SCCWRP otter trawl.
2. Epibenthos using the 3-m Gunderson beam trawl.
3. Macrobenthic infauna using the 0.2-m^2 van Veen sampler and 4-mm-mesh screen.

Phase I: Hylebos Waterway vs. Blair Waterway (1993)

Study and Sampling Design

The demersal fishes and epibenthos of the contaminated Hylebos Waterway were compared to the relatively clean and adjacent Blair Waterway in Tacoma in June of 1993 (Eaton and Dinnel, 1993) using a stratified random design. The demersal fishes and epibenthos were sampled in Phase I using the otter trawl and beam trawl, respectively. All catches were kept alive and processed onboard—catch and release sampling. Although lengths were recorded on many individuals, biomass data were not collected in Phase I.

Results of Phase I

This initial effort at defining biocriteria led to several conclusions:

- Tolerant species found in greater or insignificantly different abundance in the Hylebos Waterway compared to the Blair Waterway and confirmed by limited sampling in contaminated Eagle Harbor included:

English sole	rock Sole	sand Sole
speckled sanddab	Pacific tomcod	pygmy poacher
snake pricklebacks	sculpins (especially staghorn sculpins)	cancer crabs (especially *C. gracilis*)
coonstripe shrimp	crangon shrimp?	sea anemones
Evasterias spp. (sea star)		

- Sensitive species found in significantly greater numbers in the Blair Waterway compared to the Hylebos Waterway included:

  juvenile blackbelly eelpouts (BT) juvenile bay gobies (BT)

  sea cucumber (*Parastichopus californicus*)

- Significantly larger size categories for the Blair Waterway compared to the Hylebos Waterway included:

  male English sole purple shore crab (*Cancer gracilis*)

  Pacific tomcod, juvenile & adult

- Raw or averaged abundance and diversity data were not valuable in distinguishing the contaminated and reference sites. Length/weight regressions (WAL) for male English sole were essentially identical between the waterways.

- External health indices such as dead *Crangon* eggs, microsporidian infections, fish lesions and bloodworms (*Philometra*) were not useful in differentiating the test site from the Blair reference site.

- Five sample replications were recommended for the 7.6-m otter trawl.

Phase II: Thea Foss Waterway vs. Quartermaster Harbor (1994)

The conclusion drawn from Phase I indicated that raw or averaged abundance data were not useful in differentiating contaminated sites from reference sites. This fact led to an increased effort in recording biomass data in Phase II, and to the study of a more natural reference condition than was represented by the Blair Waterway in Phase I.

Study and Sampling Design

The coverage of the results from Phase II focused on the best-matched comparison of the QMH 1 reference station in upper Quartermaster Harbor and the contaminated TF 1 station in the upper end of the Thea Foss Waterway. The average grain size of surface sediments along the 1994 TF 1 trawl path was reported by Tetra Tech (1985) at 78% fines. This result closely matches the grain-size analysis at QMH 1 (80% fines) using the wet-sieving technique. Bottom temperatures (14 _C) were identical at both stations on 11 September 1994, and bottom salinities were closely matched with 31 ppt at TF 1 compared to 32 ppt at QMH 1. Bottom depth (MLLW) is only slightly deeper at QMH 1 (6 m) than at TF 1 (5 m). Despite these physical similarities at a point in time (late summer), it may be that the sites are not within the same class when compared seasonally. The proximity of the Puyallup River plume to the Thea Foss Waterway may contribute to annual salinity and turbidity differences unnoticed in September during low water runoff, and may reduce the validity of the comparison. It should be pointed out, also, that it is unrealistic to expect a cleanup effort in the Thea Foss Waterway to approach the biocriteria measured in Quartermaster Harbor with its larger size and natural shoreline. More valid biocriteria goals would be obtained from cleaner waterways such as the Blair or the Sitcum.

Results of Phase II

Despite these caveats, the comparison still proved to be an interesting one, and several conclusions from Phase I were reinforced in the Phase II comparison:

- Tolerant Species common to both Phase I and Phase II:

English sole	sand sole	Pacific tomcod
snake pricklebacks	Sculpins (especially staghorn sculpins)	cancer crabs (especially *C. gracilis*)
coonstripe shrimp	crangon shrimp	sea anemones
Evasterias (sea star)		

- Sensitive species common to both Phase I and Phase II:

bay goby (BT)	blackbelly eelpout (BT)	sea cucumbers

By sampling in a more natural reference area, several additional sensitive species were added to the list in Phase II:

- Sensitive species added in Phase II:

large starry flounder	pile surfperch	striped surfperch
bay pipefish	cartilaginous fishes (skates, ratfishes and dogfishes)	

Results of the second year of sampling emphasized the ecologically important fact that the reference site, despite fewer or equal numbers of fishes, supported more than twice the fish biomass than the contaminated site. Almost every fish species common to both areas was significantly larger, and fish species richness and evenness were significantly higher at the reference site. At the same time, a better comparison of impaired and reference conditions needed to be designed, one in which there would be no doubt that the reference condition was of the same physical class and supporting the same biological community as the test site.

Phase III: Sinclair Inlet, Upper Port Orchard, and Quartermaster Harbor

Objectives of Phase III

The primary objectives of the Phase III 1997 study were to test the preliminary trawl metrics developed in Phases I and II by using them in other areas, and to test their resolution by comparing a more moderately contaminated test site, Sinclair Inlet, to multiple reference sites from within the same class—Port Orchard and Quartermaster Harbor (Figure 1).

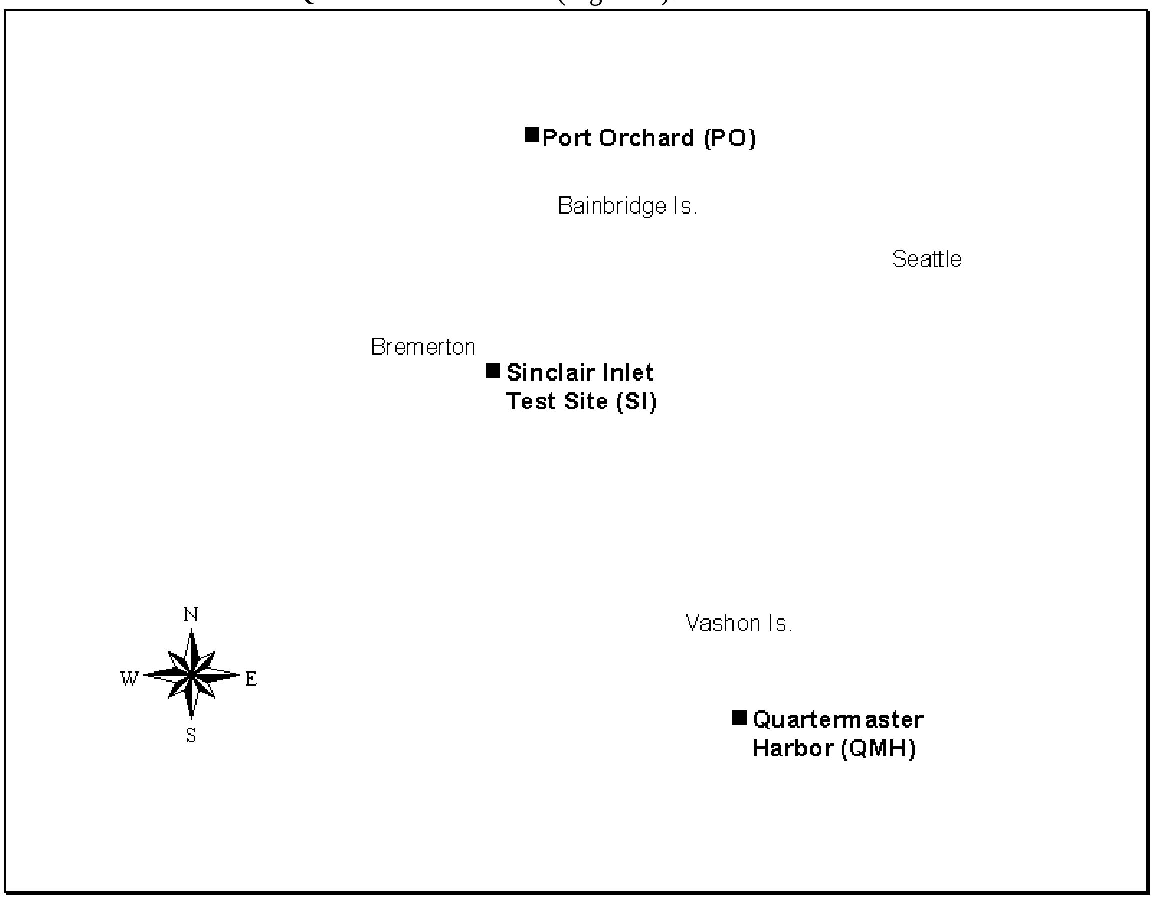

Figure 1. Study sites for Phase III. Two strata from the Sinclair Inlet test site (SI) were compared to strata from multiple reference sites of the same depth and sediment grain size in Port Orchard (PO) and Quartermaster Harbor (QMH). The benthic infaunal assemblage from QMH was not the same community as was found in the SI test site or the PO reference site. This fact precluded the use of QMH in the definition of the reference condition for Sinclair Inlet. A measurement of interannual variation in the demersal fish community was accomplished in QMH by comparing 10 replicate otter trawls from September 1997 to 11 trawls in same location from September 1994

The second objective of the 1997 field sampling was to develop another economical sampling technique for an additional biological assemblage. The macrobenthic infaunal assemblage was chosen by testing the efficacy of using 10 replicate samples per stratum with a 0.2-m^2 van Veen sampler, screening the samples through 4-mm mesh screen, and processing the samples onboard. Ten to 15 samples per day could be collected and processed using this method.

The Sinclair Inlet test site, offshore of the Puget Sound Navel Shipyard, is known to have environmental problems similar to those found in Dyes Inlet, but not to the degree of the Hylebos and

Thea Foss Waterways used in the Phase I and Phase II studies. The study area has never been dredged, and most of Sinclair Inlet still has a natural intertidal zone. The primary problem, as discussed in the PSAMP draft report from WDOE (Llanso et. al., 1998) are exceedences of the Sediment Quality Standards (SQS) for arsenic and mercury, and exceedences of the Apparent Effects Threshold (AET) for benzyl alcohol and PCBs. Eleven other compounds have been detected by the Marine Sediment Monitoring Program (MSMP) above the Effects Range-Low (ER-L) including seven other metals and total PAH. These are pollutants for which biological effects may occur occasionally (Long et al., 1995).

Johnson et. al. (1995) reported that reproductive impairment and non-fishing mortality were higher for English sole from contaminated sites in Puget Sound, including Sinclair Inlet, than from the Port Susan reference area. Mortality rates for English sole from Sinclair Inlet were surprisingly higher than those found in the more organically-contaminated Eagle Harbor Superfund site, and were comparable to those measured from the more contaminated Duwamish Waterway, despite the fact that the reported prevalence of toxicopathic disease in English sole from Sinclair Inlet was considerably lower than was encountered at either Eagle Harbor or the Duwamish Waterway (Malins et al., 1984; Johnson et al., 1988; Myers et al., 1990). Finally, the PSAMP Fish Task has reported elevated levels of lead and PCBs in tissues of English sole from the Sinclair Inlet test site (O'Neill et al., 1995).

Study and Sampling Design

- A new study design was initiated in 1997 that divided the test and reference areas into strata of approximately 350,000 m^2 whose boundaries were defined by restricted depth and sediment grain size ranges, and within which replicate samples were randomly located using Microsoft Excel's random number generator and NavTrek 97™ navigation software. Sampling took place in late summer of 1997 from August 19^{th} to September 13^{th}. Only the fish assemblage (otter trawl) and the macrobenthos (0.2-m^2 van Veen) were sampled. All samples were processed live onboard and released on stratum. Most fish species were processed as two groups—adults and subadults. Lengths and biomass data were recorded for all species—some as group weights, others as individual weights. Because of funding restraints, only two of the four initial strata were sampled:

- <u>Stratum III</u>: 11–14 m (MLLW); mean grain size = 80% fines (75%–83%); mean sediment temperature = 14.1 _C (Sinclair Inlet & Port Orchard), 13.0 _C Quartermaster Harbor; mean salinity = 30 ppt (Sinclair Inlet & Port Orchard), 31 ppt Quartermaster Harbor.

- <u>Stratum IV</u>: 14–18.3 m (MLLW); mean grain size = 73% (64%–78%) fines for Sinclair Inlet and Port Orchard, 78% fines for Quartermaster Harbor (74%–81%); mean sediment temperature = 14.1 _C (Sinclair Inlet & Port Orchard), 13.1 _C Quartermaster Harbor; mean salinity = 29.5 ppt (Sinclair Inlet & Port Orchard), 32 ppt Quartermaster Harbor.

In summary, Stratum IV is slightly deeper with a slightly coarser grain size than Stratum III. Quartermaster Harbor (QMH) was not as well matched to the Sinclair Inlet (SI) test site as was Port Orchard (PO), because Quartermaster Harbor had slightly finer sediments in Stratum IV (78% vs. 73%), slightly colder temperatures (13.1 _C. vs. 14.1 _C.), and slightly higher bottom salinities. It was also immediately apparent upon sampling the benthic infauna that Quartermaster Harbor, despite its reasonably well matched physical parameters to Sinclair Inlet and Port Orchard, did not represent a comparable biological community—the ultimate test of the physical classification. The deposit-feeding bivalve molluscs, so prominent in our samples from Sinclair Inlet and Port Orchard, were almost absent from the QMH samples. No burrowing anemones (*Pachycerianthus*) or sipunculids (*Golfingia*) could be found. Large deposit-feeding bamboo worms (maldanidae) dominated the QMH samples but were extremely rare in the SI and PO samples. This lack of a good pairing frustrated one of the primary objectives of the Phase III sampling: to compare the Sinclair Inlet test site to **multiple** reference sites from within the same class. On the other hand, the upper Port Orchard reference site represented the best pairing to a test site ever obtained in the three phases of the biocriteria/bioassessment sampling. Therefore, the results of the Sinclair Inlet/Port Orchard pairing will be emphasized in the discussion, along with the interannual variation in the Quartermaster Harbor demersal fish sampling from September 1994 versus September 1997.

The voluminous population data were entered into Excel 7.0 spreadsheets and then initially scanned as bar graphs. This data was then transferred to a statistical software package, SPSS/Windows, where potentially interesting patterns were graphed as box plots to help visualize the distribution of the variable (Figures 2–5). Box plots were chosen because of their ability to visually represent the central tendency of a range of data generated by replicate sampling. The box itself, called the interquartile range (IQR), represents 50% of all cases and extends from the 25th to the 75th percentile with the horizontal line representing the median value (50th percentile). The vertical lines (whiskers) are drawn to the largest and smallest values that are outside the box but within 1.5 box lengths, and represent the range of data not considered to be outlying or extreme. Outliers (o) lie within 1.5–3 box lengths from the upper and lower edges of the box, and extreme values (*) are more than three box lengths from the upper and lower edges. The central tendency is represented by the median (the horizontal line within the box), the spread or variability by the length of the box and whiskers, and the symmetry of the spread by the position of the median line within the box. If the median line is closer to the bottom of the box than to the top, there is a tail toward the larger values (positive skewness) or vice versa, with the length of the tail shown by the length of the box and the length of the whiskers and the outlying and extreme values. The biocriteria are often defined visually by the box plots themselves, with the lower edge of the box (the 25th percentile) for the displayed metric variable from the reference areas serving as the goal or criterion. In a monitoring or cleanup effort, the target median of the test site variable or index would be this 25th percentile reference value.

Results of Phase III

Demersal Fish Populations

Many of the premises developed in the Phase I and Phase II sampling were confirmed in the comparison of the Sinclair Inlet test site to the upper Port Orchard reference site (Table 1, Figures 2–3) including the following:

- Fish biomass was much lower at the test site compared to the reference site (Figure 2).
- Flatfish biomass was much lower at the test site compared to the reference site (Figure 2).
- Starry flounder, cartilaginous fishes, subadult flatfish, and Pacific herring biomass and abundance were greatly reduced at the Sinclair Inlet test site (Figure 2, cont.).
- English sole, sand sole, the common sculpins (especially staghorn sculpin) and juvenile (subadult) Pacific tomcod, snake pricklebacks and shiner surfperch were all confirmed to be tolerant species.
- Starry flounder, cartilaginous fishes, and giant sea cucumber once again appeared to be sensitive species.
- Tolerant flatfish abundance and biomass (especially English sole) were numerically slightly higher and more variable at the test site than at the reference site (Figure 3).
- Common sculpins (staghorn, northern, and roughback) abundance and biomass were elevated at the test site relative to the reference site (Figure 3).

In addition to the confirmed premises above, the following additional characteristics that had not been previously noted in Phases I and II separated the test site from the Port Orchard reference site:

- Total fish abundance was reduced at the test site (Figure 2).
- Flatfish abundance was reduced at the test site (Figure 2). Previously, abundance data had not been useful in differentiating sites.

- Subadult flatfish abundance and biomass were greatly reduced at the test site (Figure 2, cont.).
- Rock sole, speckled sanddabs, and plainfin midshipmen rejoined the list of tolerant species where they had been placed during Phase I.
- Adult Pacific tomcod, snake pricklebacks and shiner surfperch (unlike their subadults) were reduced at the test site. This may have been first noted this year because of the additional effort of separating adult and subadult individuals during onboard processing.
- Adult bay gobies from the otter trawl catches were numerically more abundant at the test site than at the reference sites, and should be considered a tolerant group. In past sampling, juvenile bay gobies (sampled in the beam trawl, not used in Phase III) were significantly reduced at the impaired sites (Hylebos and Thea Foss Waterways) and are still considered a sensitive indicator.
- Even though flatfish biomass (Figure 2) and flatfish mean individual weights were significantly lower at the impaired site compared to the reference sites, the mean individual weights of adult English sole (Figure 3) and rock sole from Sinclair Inlet were higher than those from Port Orchard. This may reflect a release from competition with large starry flounder which were almost absent from the test site and which make up the bulk of the flatfish biomass from Port Orchard.
- Although adult Pacific tomcod were numerically reduced at the test site compared to both reference sites, the mean individual weights of these adults were much higher at the test site, unlike the results from the Phase I and Phase II studies.
- The derived metrics of richness, dominanc, and evenness displayed no significant differences between the test site and the Port Orchard reference site (Figure 4), as was also found in the Phase I comparison but not in Phase II.

Table 1. A summary evaluation of trawl metrics and the apparent response to environmental impairment.

Apparent Response to Impairment

BT = Beam Trawl, epibenthos, and small fishes

OT = Otter Trawl, larger demersal fishes

Candidate Metric	**1993:** **Hylebos vs. Blair Wtys.** Caveat: Hylebos has much more wood debris than Blair	**1994:** **Thea Foss vs. QMH** Caveat: QMH may not be an ideal ref. for Thea Foss	**1997:** **SI vs. Port Orchard** The best comparison	**1998:** **Predicted Response:**	**Useful Metric?**
Demersal fish abundance per unit area (OT)	No significant difference	Increase or no difference	Decrease	Decrease or no difference	?
Demersal fish biomass per unit area (OT & BT)		Decrease	Decrease	Decrease	Yes
Total flatfish biomass (OT)		Decrease	Decrease	Decrease	Yes
Subadult flatfish abundance and biomass (OT)			Decrease	Decrease or no difference	Yes
Starry Flounder abundance & biomass (OT)		Decrease	Decrease	Decrease	Yes
Rock Sole abundance and biomass (OT)	No significant difference	Decrease	No significant difference	No difference	No
English Sole abundance & biomass (OT)	No significant difference	Increase	Increase	Increase or no difference	No
Bay Goby abundance and biomass (BT)	Decrease	Decrease		Decrease	Yes
Cartilaginous fish abundance and biomass (OT)		Decrease	Decrease	Decrease	Yes
Pacific Staghorn Sculpin abundance and biomass (BT)	Increase	Increase	Increase (OT)	Increase	Yes
Subadult Pacific Tomcod abundance & biomass (OT)	No significant difference	Increase	No significant difference	No significant difference	No
Adult Pacific Tomcod abundance & biomass (OT)	No significant difference	No significant difference	Adults Decrease?	Decrease or no difference	?
Fish Mean individual weights	Depends on species	Decrease	Depends on species	Depends on species	No
Fish Weight / Length Ratio	No significant difference		No significant difference	No significant difference	
Pacific Staghorn Sculpin mean individual weights (OT)		Increase	No significant difference	No significant difference	No
Adult Shiner Surfperch abundance and biomass (OT)		Increase or no difference	Decrease	Decrease or no difference	No
Subadult Shiner Surfperch abundance and biomass (OT)			Increase	Increase	?
Snake Prickleback abundance (OT)	No significant difference	No significant difference	Decrease	No significant difference	No
Snake Prickleback abundance (BT)	Increase	No significant difference	No data	No significant difference	No
Adult Snake Prickleback abundance (OT)			Decrease	Decrease or no difference	No
Subadult Snake Prickleback abundance (OT)			No significant difference	No difference or increase	No
Sea cucumber abundance and biomass (OT&BT)	No data	Decrease	Decrease	Decrease	Yes

Table 1 (continued). A summary evaluation of trawl metrics and the apparent response to environmental impairment.

Apparent Response to Impairment

BT = Beam Trawl, epibenthos and small fishes
OT = Otter Trawl, larger demersal fishes

Candidate Metric	**1993: Hylebos vs. Blair Wtys.** Caveat: Hylebos has much more wood debris than Blair	**1994: Thea Foss vs. QMH** Caveat: QMH may not be an ideal ref. for Thea Foss	**1997: SI vs. Port Orchard** The best comparison	**1998: Predicted Response:**	**Useful Metric ?**
Sensitivity Index (Sensitive / Sensitive + Tolerant)	Decrease	Decrease	Decrease	Decrease	Yes
Total species richness	No significant difference	Decrease or no difference	No difference	No difference	No
Dominance and Evenness	Not analyzed	Decrease or no difference	No significant difference	No significant difference	No
External fish abnormalities	No significant difference	No significant difference	No significant difference	No significant difference	No
Crustacean abundance and biomass (BT)	Increase	Increase	No data	Increase	Yes
Cancer crab abundance and biomass (BT)	Increase	Increase	No data	Increase	Yes
Cancer gracilis abundance and biomass (BT)	Increase	Increase	No data	Increase	Yes
Evasterias troschelli (mottled seastar) abundance (BT)	Increase	Increase	No data	Increase (debris-dependent)	No
Pile anemone abundance and biomass (BT)	Increase	Increase	No data	Increase (debris-dependent)	No
Mean Individual Weights:					
Adult Flatfish as a group	No data	Decrease	Decrease	Decrease	Yes
Adult English Sole	Decrease	Decrease	Increase	Unknown	No
Adult Rock Sole	No significant difference	Decrease	Increase	Unknown	No
Shiner Surfperch	No data	Decrease	Increase	Unknown	No
Adult Pacific Tomcod	Decrease	Decrease	Increase	Unknown	No
Staghorn Sculpins	No significant difference	Increase	No significant difference	No significant difference	No

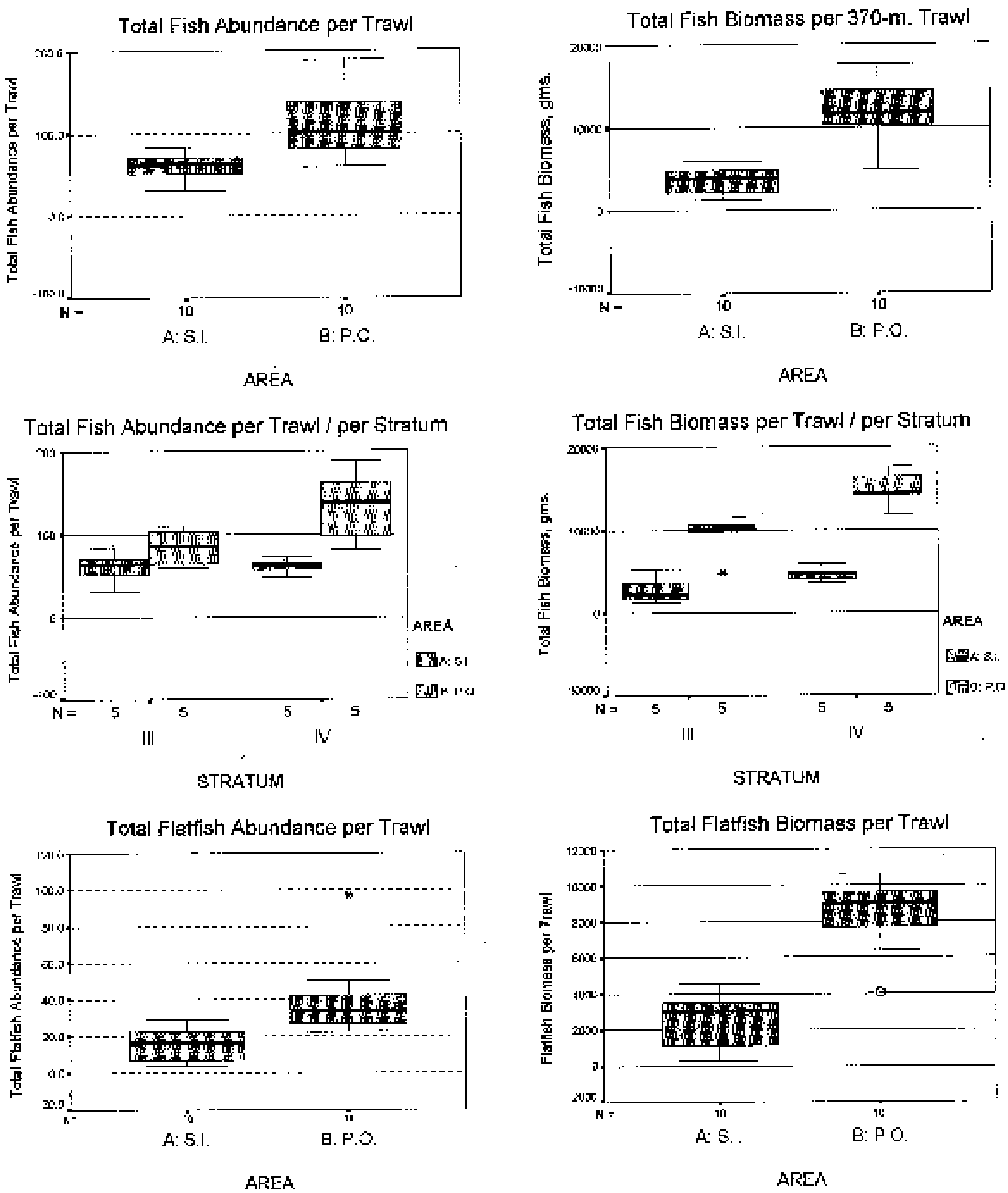

Figure 2. Comparisons of fish abundance and biomass metrics between the Sinclair Inlet (SI) test site and the Port Orchard (PO) reference site. Results were either combined for the two strata (n=10) or the strata were analyzed separately (n=5). Note the wider separation of the biomass data between the test and reference site compared to the abundance data. All fish trawls were 370 m long at 2.5 knots.

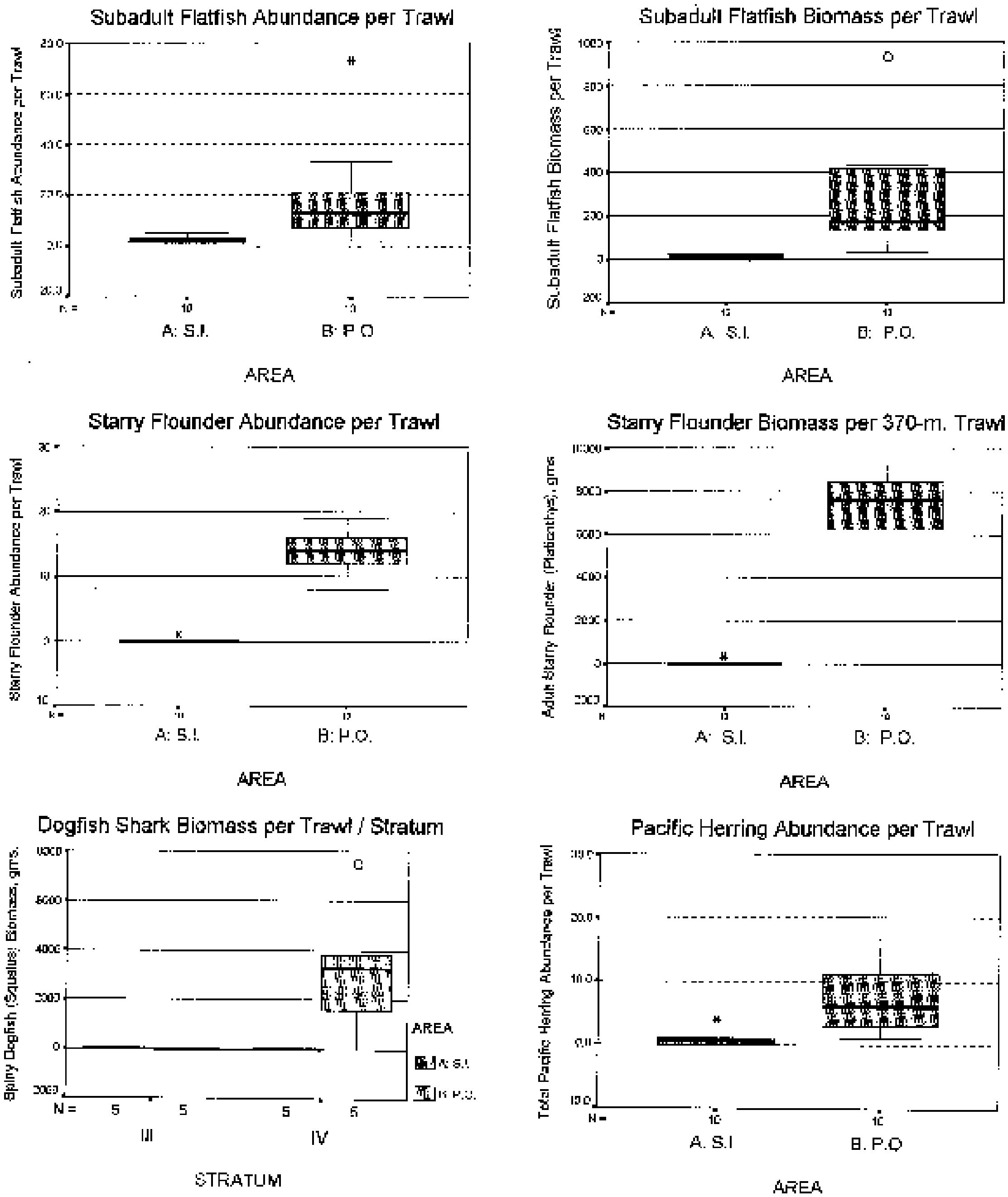

Figure 2 (cont.). Comparisons of fish abundance and biomass metrics between the Sinclair Inlet (SI) test site and the Port Orchard (PO) reference site. Subadult flatfish (<150 mm long), adult starry flounder, and Pacific herring were almost absent from the test site in Sinclair Inlet. Spiny Dogfish (*Squalus acanthias*) were not encountered in Sinclair Inlet, nor in stratum III of the reference area (PO). All fish trawls were 370 m in length at 2.5 knots.

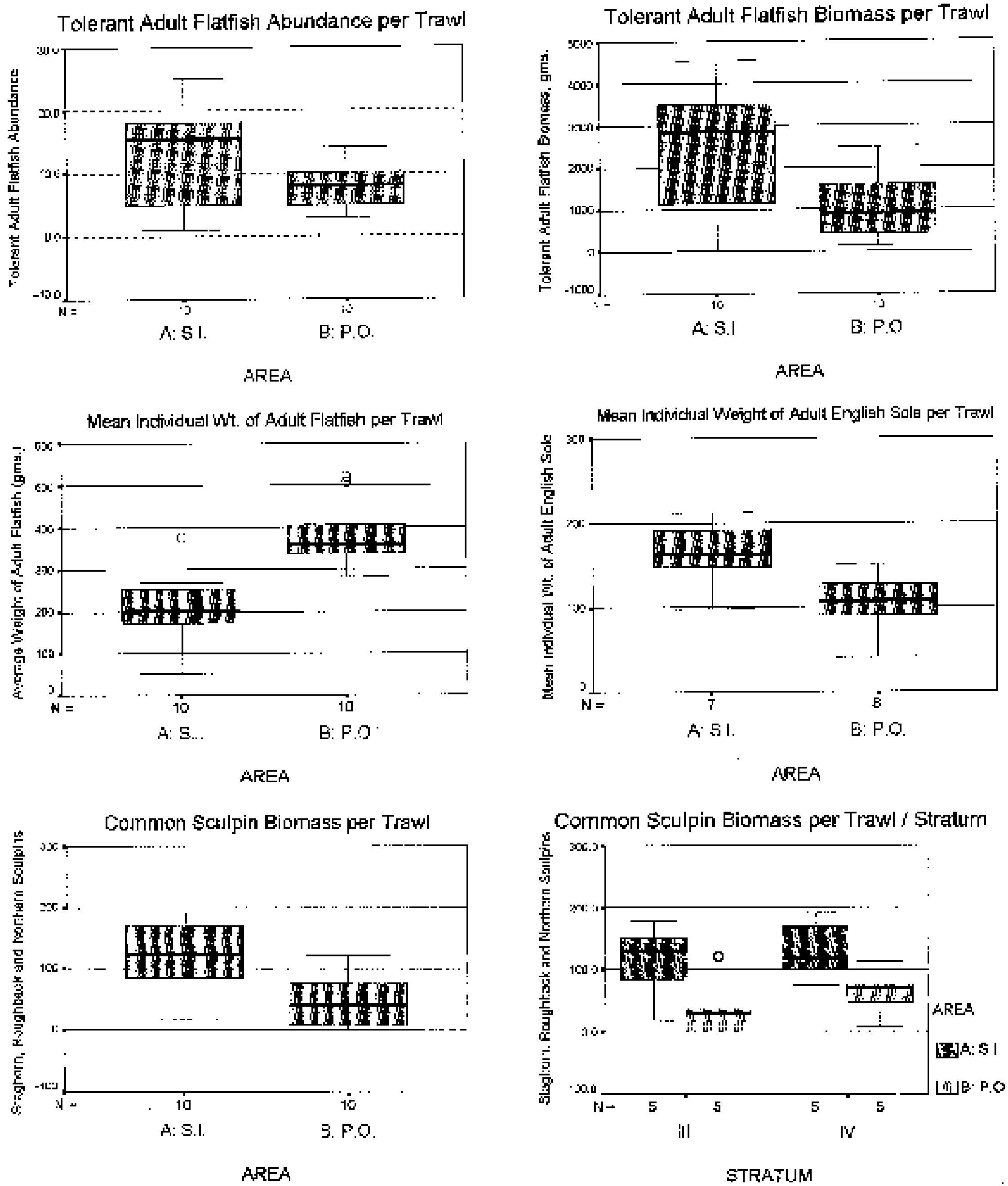

Figure 3. Some tolerant fish categories. Tolerant flatfish species (all flatfish except subadults and starry flounder) were numerically more abundant and more variable at the Sinclair Inlet test site. Although the mean individual weights of adult flatfish as a group was much higher at the Port Orchard (PO) Reference site (primarily because of starry flounder), the mean individual weight of adult (>150 mm) English sole was higher at the Sinclair Inlet (SI) test site. The common sculpins (primarily staghorn sculpins) seemed to flourish at the test site as was the case in the Hylebos and Thea Foss Waterways Superfund sites during the Phases I and II studies. All fish trawls were 370 m in length at 2.5 knots.

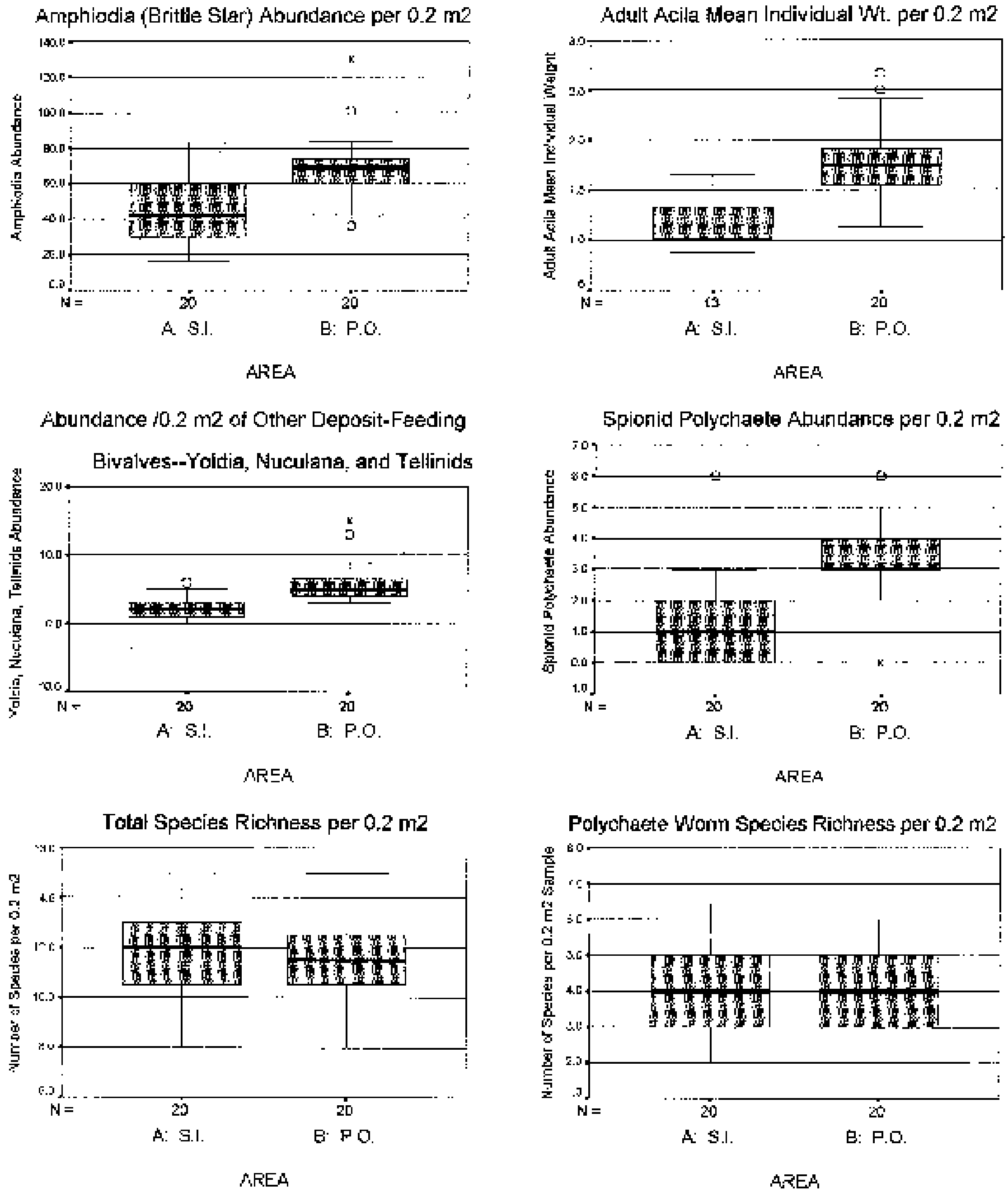

Figure 4. Some metrics tested for the macrobenthic infaunal assemblage in Phase III. Brittle star (*Amphiodia urtica*) abundance, adult nut clam (*Acila castrensis*) mean individual weights (adults and juveniles were weighed separately as groups), and the abundance of spionid polychaetes and other deposit-feeding bivalves appear to have some promise as useful metrics in separating the Sinclair Inlet test site from the Port Orchard reference site. The derived metrics of species richness, dominance and evenness were not useful at the level of the 4-mm screens. All samples were taken with a 0.2-m^2 van Veen sampler, sieved through 4-mm screens, and processed onboard.

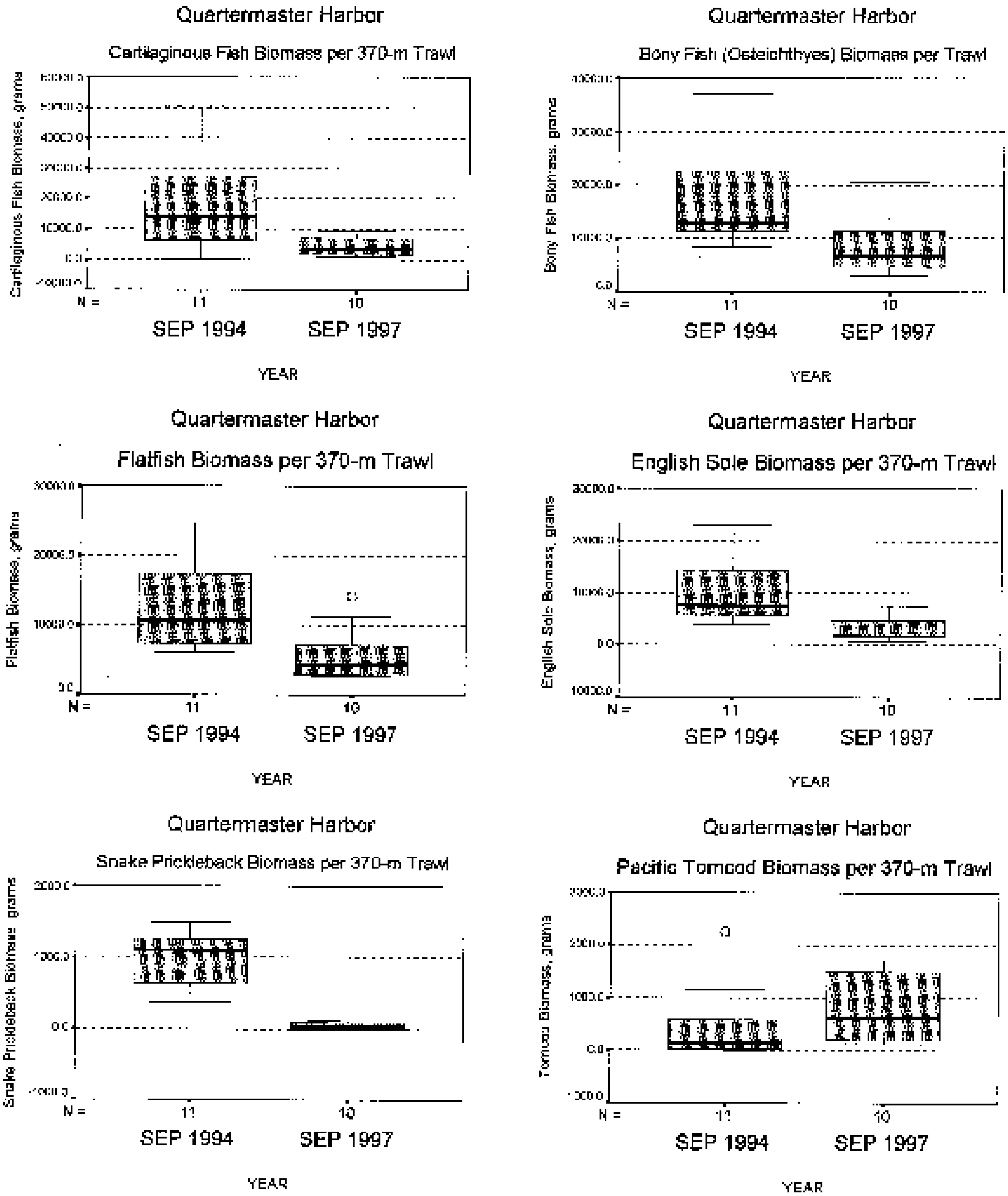

Figure 5. Comparisons of three-year, interannual variation in demersal fish biomass categories, September 1994 and September 1997, mid-Quartermaster Harbor. Most categories declined over the three-year period, although some categories showed no change (spiny dogfish, rock sole, flatfish mean individual weights), and a few actually showed a numeric increase (starry flounder, Pacific tomcod, and Pacific herring). This means that three-year-old demersal fish data cannot be used for comparisons to contemporary data.

Macrobenthos

The macrobenthic infaunal assemblage was sampled with a 0.2-m^2 van Veen sampler and screened through 4-mm mesh screens (Figure 4). Ten random replicate samples were taken per stratum for a total processed sample of 2.0 m^2. All retained invertebrates were processed onboard. Polychaete identification was aided by the expert advice of Kathy Welch from WDOE/EILS. All animals were identified and counted, and larger species, mainly bivalves, were also weighed. Brittle stars (*Amphiodia*) were counted on the screens (bodies only). Ten to 15 samples per day could be collected and analyzed. Temperature, salinity, and sediment grain-size data were also assessed in each sample.

Potentially valuable metric variables (Figure 4) from the 4-mm mesh screens that were numerically **higher** at the Port Orchard reference sites include the following (remember that the mean size of most of these species is larger than for those retained on 1-mm screens):

- Brittle star (*Amphiodia*) abundance;
- Nut clam (*Acila castrensis*) biomass and mean individual weights;
- Other deposit-feeding bivalve abundance (*Yoldia*, *Nuculana*, and tellinids);
- Spionid polychaete abundance; and
- Capitellid polychaete abundance.

Potentially valuable metric variables from the 4-mm mesh screens that were numerically lower at the Port Orchard reference sites include the following:

- Chaetopterid polychaete abundance;
- Goniadid and glycerid polychaete abundance;
- Burrowing anemone (*Pachycerianthus*) abundance; and
- Ribbon worm (Nemertea) abundance.

Potential metric variables that showed no promise in discriminating between test and reference sites:

- All taxa-richness categories; and
- Polychaete total abundance.

Temporal Demersal Fish Variation in Quartermaster Harbor, September 1994 to September 1997

In order to study the kinds of temporal variation one might expect in demersal fish data collected over a three-year time span, 10 replicate trawls from strata III and IV from mid-September, 1997 were compared to the 10 replicate trawls taken in the same area using the same gear and boat during mid-September, 1994. No commercial fishing had taken place in Puget Sound during the three-year period. The results, summarized in Figure 5, include the following:

Declining Categories:

- Total numeric fish abundance and biomass in 1997 was <40% of that encountered in 1994.
- Flatfish abundance and biomass decreased by about 50%, with all species except starry flounder and rock sole showing dramatic declines. English Sole abundance declined by 67%.
- Cartilaginous fish abundance declined by 77%, all of which was represented by the spotted ratfish.
- Shiner surfperch (78%), staghorn sculpins (51%), and snake pricklebacks (94%) all displayed large numeric declines in abundance.

Increasing Categories:

- Both adult and subadult Pacific tomcod increased by more than 200%.
- Pacific herring displayed a 150% numeric increase.
- Starry flounder increased by <300%, whereas rock sole showed no change.

Discussion

The data from the 1997 sampling period (Phase III) definitely represents the best-paired comparison of test and reference data from the three phases of the Puget Sound biocriteria developmental research, with the data from Phases I and II both hindered by mismatches. The Hylebos Waterway test site in Phase I contained an abundance of woody debris not found in the Blair Waterway reference, which may have complicated the comparisons. The Quartermaster Harbor reference comparison to the Thea Foss Waterway, although showing comparable physical parameters in September, may indeed be of another class when physical parameters are sampled continuously or monthly. In Phase III, it was hoped to compare the test site, Sinclair Inlet, to two reference sites, upper Port Orchard and mid-Quartermaster Harbor. Although the physical parameters were well matched, the benthic infaunal community of Quartermaster Harbor obviously did not represent the same biological community as was encountered in Sinclair Inlet and Port Orchard. For this reason, the main emphasis was placed on the comparison of these last two areas, and the Quartermaster Harbor sampling was used primarily to study the temporal variation in the demersal fish community between September, 1994 and September, 1997. Of course all of these efforts provide valuable lessons for applying biocriteria to Puget Sound, which are summarized in Table 1. The predicted metric response to impairment is based on the history of the Puget Sound biocriteria research, with the Phase III study given highest priority, followed by Phase I and then Phase II. The recommendations for studying and incorporating data from the three biological assemblages follows:

Demersal Fish, 7.6-m Otter Trawl

The demersal fish community seems especially sensitive to environmental impairment in Puget Sound and can be easily and economically sampled with live, onboard processing. With the methods developed over 18 years of research trawling, up to eight trawls per day can be collected and processed with very little mortality. The added emphasis of separately processing adults and subadults of most species has added increased refinement to the evaluation and efficacy of the candidate metrics.

Demersal fish metrics that seem especially powerful for differentiating impaired conditions from reference, and whose response to environmental impairment is noted, include the following (Table 1):

Total fish biomass	reduced
Total flatfish biomass	reduced
Total adult flatfish mean individual weights	reduced
Total cartilaginous fish biomass	reduced
Starry flounder biomass	reduced
Pacific herring abundance and biomass	reduced
Common sculpin abundance and biomass	elevated
Tolerant fish categories (all flatfish except starry flounder, subadult shiner surfperch, subadult Pacific tomcod, subadult snake prickleback, staghorn sculpin, roughback sculpin, northern sculpin)	elevated or no significant difference

Demersal fish metrics that have good, but as yet unproved potential include the following:

Subadult flatfish abundance	reduced
Adult Pacific tomcod, adult shiner surfperch and adult snake prickleback abundance and biomass	reduced
Sensitive Category Index	reduced

Demersal fish metrics for which potential has been reduced by the Phase III sampling include:

- Mean individual weights of individual flatfish and gadid species (complicated by the presence/absence of competitors);
- Weight-length regressions (WAL) for adult flatfish species—the only correlations detected were to size class and not to site or stratum; and
- Derived metrics such as richness, dominance and evenness.

Demersal Fish Metrics over Time: QMH ’94 vs. QMH ’97

Although more cases of temporal variation need to be documented, the precipitous change in most metrics from two of the Quartermaster Harbor strata between September of 1994 and September of 1997 (Figure 5) does not bode well for anything but synoptic sampling of reference and test sites for this biological assemblage. The epibenthos (3-m beam trawl) were not sampled in 1997, so no conclusions can as yet be drawn concerning the temporal variation of this assemblage. Continued late-summer sampling of all the QMH strata are recommended to establish trends and more data on temporal variation.

Epibenthic Invertebrates and Small Fishes, 3-m Gunderson Beam Trawl

Although the epibenthos was not sampled in Phase III, the earlier phases provided a wealth of data and lessons. In Phase I, the best information came from the beam trawl, despite the difficulty of using the gear in a waterway with abundant wood debris. Future recommendations for sampling with this gear would include shortening up the beam trawl tows to 100 m in length and increasing the number of random replicate samples to five.

Epibenthic metrics from the beam trawl showing the greatest potential for differentiating impaired from reference conditions include (Table 1):

Sensitive fish categories (juvenile bay goby, juvenile blackbelly eelpout)	reduced
Total fish abundance	reduced
Total fish species richness	reduced
Subadult snake prickleback abundance	elevated
Staghorn sculpin and total sculpin abundance and biomass	elevated
Giant sea cucumber (*Parastichopus*) abundance	reduced
Total crustacean abundance and biomass	elevated
Cancer gracilis abundance and biomass	elevated
Sensitive Category Index	reduced

Macrobenthic Infauna on 4-mm Mesh Screens

The goal for the development of metrics for the macrobenthos was to increase the economy of the data derived from this biological assemblage to more closely match the data from the demersal fish and epibenthic assemblages. By increasing the screen mesh size (from 1.0 mm to 4.0 mm) as well as the sampler size (from 0.1 m^2 to 0.2 m^2) and the number of replicates (from 5 to 10), and then using onboard processing, the goal was to derive useful complimentary metrics at one-tenth the cost of metrics derived from 1.0-mm screens. The samples used for the macrobenthos need to be collected in any case for measurements of the physical environmental factors including sediment grain size, sediment temperature, and salinity of the overlying water. Future research recommendations for the development of the infaunal metrics would include the trial of screen mesh intermediate between the 4-mm and 1-mm screens.

Potentially valuable metric variables from the 4-mm mesh screens that were numerically **higher** at the Port Orchard reference sites include the following:

- Brittle star (*Amphiodia*) abundance;
- Nut clam (*Acila castrensis*) biomass and mean individual weights;
- Other deposit-feeding bivalve abundance (*Yoldia*, *Nuculana*, and tellinids);
- Spionid polychaete abundance; and
- Capitellid polychaete abundance.

Potentially valuable metric variables from the 4-mm mesh screens that were numerically lower at the Port Orchard reference sites include the following:

- Chaetopterid polychaete abundance;
- Goniadid and glycerid polychaete abundance;
- Burrowing anemone (*Pachycerianthus*) abundance; and
- Ribbon worm (Nemertea) abundance.

Summary

The three phases of the biocriteria/bioassessment developmental research have attempted to construct useful metrics derived from population patterns and health indices of three different marine biological assemblages. Each assemblage and sampling technique represents a different and complimentary assessment scale from the largest and most-mobile demersal fish scale (370-m trawls) to the smallest and least-mobile macrobenthic invertebrate scale (0.2-m^2). Random replicate sampling and the comparison of central tendencies to reference value benchmarks (the biocriteria) lies at the heart of the biocriteria/bioassessment methods. By scoring each metric based on its relative distance to the reference value benchmark and combining the scores into an index for each biological assemblage, it is hoped that both economy and ecological meaning can be restored to the assessment and cleanup of environmental impairment.

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